DIAGNOSIS OF CANDIDEMIA: EVALUATION OF THE B-GLUCAN WAKO TURBIDIMETRIC TEST VERSUS FUNGITELL COLORIMETRIC TEST FOR THE DOSAGE OF 1-3 ß-D-GLUCAN

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Fig.1

Introduction: Invasive Candidiasis (CI) is the most common Invasive Fungal Infection (IFI) in non-neutropenic patients. The diagnosis is traditionally based on blood culture, which however requires several days of incubation (Fig.1) and in any case with limited sensitivity which does not exceed 70%. For this reasons, several biomarkers have been studied to favor a faster diagnosis and become part of the anti-fungal stewardship. International guidelines recommend the use of (1-3) -β-D-Glucan (BG), a test with high negative predictive value (NPV). From April 2018 three commercial systems are now certified for diagnostic use, and all of them are currently available in Italy: Fungitell (Associates of Cape code Corprate), β-Glucan test (FUJIFILM Wako Chemicals Industries), Goldstream Betaglucan (ERA Diagnostics).

To date, all BG diagnostic performance data have been obtained using the Fungitell assay. The recent test launched in Europe to detect BG, the FUJIFILM Wako β-glucan test, use different reference standards than Fungitell (pachiman versus lentinan). It differs also in the detection technique, turbidimetric instead of colorimetric and Cut-off value.

Objective: Aim of this retrospective study was to evaluate the diagnostic performance of the Wako β-glucan test kit (WAKO) compared to the Fungitell test (FUNGITELL) in the diagnosis of Invasive Candidiasis. The key objective was the optimization of the cut-off considering also technical user-friendly of the test.

Materials and Methods:

All patients presenting between January 2013 and May 2018 with BC-proven candidemia and an archived serum sample from the day of BC sampling (± 2 days) were included. BC diagnostic was performed using the BacT/Alert 3D microbial detection system (bioMérieux, Marcy l'Etoile, France). Sera from control group consisted of patients with negative BC were examined.

BG detection was performed using the Fungitell kit according to the manufacturer's instructions (Fig.2) and using the Wako β-glucan kit. In short, 100 µL of serum were used for the Wako test, to which 900 µL of ready-to-use pre-treatment solution were added and subjected to incubation at 70 ° C for 10 minutes; after cooling in ice 200 µL of treated serum were added to the dehydrated LAL reagent and placed in gelation reader (MT-5500 toxinometer) at 37 ° C. Gelation of the LAL was measured for a maximum of 90 min (FIG 3). The BG concentration in each sample was calculated comparing the result with a calibration curve specific to each lot and supplied by the manufacturer. Performance was calculated, considering the cut off of ≥80 pg / mL for Fungitell and ≥11pg / mL for Wako, as indicated by the manufacturer and then analysing different cut off: 150 pg / mL for Fungitell and 8, 6 and 4 pg / mL for Wako.

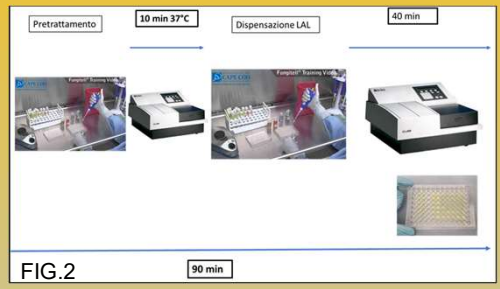

FIG.2

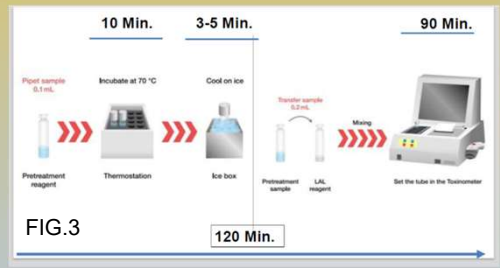

FIG.3

TAB.1

	Performance test BG			
	Sens	Spec	VPP	VPN
WAKO ≥11pg/ml	77	77	72	81
WAKO ≥8pg/ml	82	70	68	84
WAKO ≥6pg/ml	86	67	66	86
WAKO ≥4pg/ml	95	48	58	93
Fungitell ≥80 pg/ml	98	43	56	98
Fungitell ≥150pg/ml	86	71	69	87

TAB.2

	FUNGITELL	WAKO
METHOD of DETECTION	Colorimetric	Turbidimetrico
SAMPLE	Serum	Serum/plasma
VOLUME OF SAMPLE	5 µl	100 µl
REAGENT	LAL	LAL
TYPE OF SUPPORT	96 well-plate	Single tube
PRETREATMENT	10 min at 70°C	10 min at 70°C and then 3 min at 0°C
CUT-OFF	80 pg/ml	11pg/ml
TIME OF DETECTION	40 min	90 min

Results: 176 patients enrolled at the Verona University Hospital were evaluated. Patients with positive blood culture for Candida spp. were 75, for a total of 88 candidemic episodes. Patients enrolled in control group were 101 for a total of 115 episodes with negative blood culture. The two tests were concordant in 87% of cases if the cut offs of 80pg / mL for Fungitell and> 4 pg / mL for Wako were considered. Results with different cut-off are reported in Tab.1.
Technical performance detected for the two tests are reported in Tab.2 and Fig.2 and 3.

Conclusion: Best performance of Wako test was obtained with a lower cut off of 4 pg/mL. Accuracy was similar to Fungitell. Technical procedure of Fungitell test, because of serum volume and reagents that need to be prepared, requires expert operators and dedicated execution times of around 30-40 minutes, to perform up to 23 samples in duplicate in 90 minutes. Wako test, which all reagents are ready for use in single tubes, use higher volumes of serum than Fungitell and does not require expert personnel. Execution time is approximately 120 minutes for 16 samples, with an operator time of about 20 minutes. Given the high specificity shown for BG values close to 11 pg / mL , WAKO is an excellent system to exclude invasive Candidosis but also to support the indirect diagnosis of invasive candidiasis. Furthermore, Wako's simple procedure and single-tube packaging make it a valuable tool for urgent requests as well as for small hospitals with unskilled personnel or with reduced volumes of activity.

References:
Friedrich R, Rappold E, Bogdan C, Held J. 2018. Comparative analysis of the Wako β-glucan test and the Fungitell assay for diagnosis of candidemia and *Pneumocystis jirovecii* pneumonia. J Clin Microbiol 56: e00464-18. https://doi.org/10.1128/JCM.00464-18.